

The University of Chicago

THE DEVELOPMENTAL CAPACITY OF
THE UNINCUBATED CHICK BLASTO-
DERM AS TESTED IN CHORIO-
ALLANTOIC GRAFTS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1934

By
ELIZABETH BUTLER

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THE DEVELOPMENTAL CAPACITY OF REGIONS OF THE UNINCUBATED CHICK BLASTODERM AS TESTED IN CHORIO-ALLANTOIC GRAFTS

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ONE TEXT FIGURE AND THREE PLATES (NINE FIGURES)

INTRODUCTION

Using the medium of the chorio-allantoic graft, Willier and Rawles ('31 a) and Hunt ('31, '32) have shown for the chick blastoderm of the definitive streak stage, that the region which has the capacity to differentiate practically all embryonic parts is the primitive node and its immediate vicinity. This led us to the work reported in this paper, which is an attempt to trace the location of a corresponding area in the unincubated pre-streak blastoderm. Preliminary experiments revealed that the whole unincubated blastoderm, when transplanted, had the power to produce approximately all parts of an embryo. Consequently, further experiments were designed to determine the exact location of the area capable of such differentiation. This necessitated, first, a study of the structure of the unincubated blastoderm with special reference to its axial relations. With known orientation, the blastoderm was then cut into various pieces, viz., anterior and posterior halves, right and left halves, etc., and grafted to the chorio-allantois. It was found that only those pieces which contained the intact median posterior quadrant possessed the capacity to form the various tissues of the embryo. This region is thus found to correspond in its powers of development to the node field of a definitive streak stage. A preliminary account of the findings was given before the Society of Zoölogists (Butler, '33).

It is with a sense of deep appreciation that I express my thanks to Dr. B. H. Willier for suggesting this problem and also for his helpful and stimulating guidance throughout its progress.

STRUCTURE OF THE UNINCUBATED BLASTODERM

Before taking up the methods used, it seems best to pause for a brief consideration of certain features in the structure of the pre-streak blastoderm that could be used in ascertaining the anteroposterior axis. After removal of the yolk from the living blastoderm, it can be seen in a great many cases, that the entoderm has not reached the anterior and anterolateral germ wall. The same situation is noted, too, in a considerable number of blastoderms prepared as whole mounts. There is much variability in the extent to which the entoderm has advanced anteriorly, as reflected in the greater or lesser extent of an anterior less opaque region lacking the under layer. In some cases, the entoderm extends only just beyond the center, while in others, this layer has reached the anterior limits of the pellucid area, but has not as yet formed a coherent layer in that region (fig. 2). In still other cases, it is impossible to determine the orientation of the blastoderm, since the entoderm forms a complete layer and the pellucid area appears homogeneous throughout (fig. 3). It is thus evident that the term 'unincubated' as used in this paper covers a series of stages in the forward extension of the entoderm, prior to the appearance of the streak.

That the anterior area free of entoderm is not the result of tearing the delicate under layer by careless manipulation is checked in two ways. First, careful observation during the removal of the yolk reveals that, in general, the subgerminal cavity adequately separates the blastoderm from the yolk, at least over the pellucid area. Since there is no intimate contact in this region, it is quite possible to remove the blastoderm without disturbing the entoderm. In those cases where tearing definitely has occurred, the condition is

quite obvious, since the edges of the torn area are raised and ragged. Second, a study of blastoderms fixed on the yolk and serially sectioned corroborates the observations above relative to variability in the anterior extent of the entoderm, and the under surface of such blastoderms has been protected throughout by a relatively thick layer of yolk. Similarly, His (1868), Patterson ('10), Lillie ('27), and Wetzel ('29, '32), all agree that, in general, prior to incubation, a complete layer of entoderm is not found.

However, no evidence was found for an 'embryonic shield' based, according to some authors, mainly on variation in thickness of the upper layer. It is true that the height of the meso-ectoderm varies somewhat, but never is there a modification in height sufficiently sharp to account for a 'shield' in surface view. It would seem that the variation in anterior extent of the entoderm offers the principal basis for the appearance of a more opaque area posteriorly. Certain authors, Waldeyer (1869) and Koller (1882), have related the appearance or non-appearance of the 'shield,' respectively, to the incomplete or complete layer of entoderm.

His also mentions the importance of conditions in the underlying yolk as a factor in determining the appearance of the pellucid area in surface view. Likewise, Mitrophanow (1899), Wetzel ('29), and Dehnel ('29) suggest that the appearance of the blastoderm while on the yolk is in part due to irregular heaps of yolk beneath the pellucid area, to the white yolk of Pander's nucleus, or to variation in the depth of the subgerminal cavity. During the many observations made for these experiments, it was noted that certain of these extra-blastodermal features play a considerable role in the appearance of the blastoderm on the yolk.

In the series of stages studied, the streak first appears at the posterior limit of the pellucid area as a very short structure, broad in proportion to its length (fig. 4). Its length, at first about one-fourth of the diameter of the pellucid area, increases in later stages and the streak becomes narrower in proportion to its length, though it is never very

broad in relation to the whole blastoderm. At no stage is there a resemblance to the 'broad primitive streak' described by Hoadley ('26 a, b) as always present after 2 hours of incubation. It is also shorter than the stage Waldeyer (1869) first described as the broad primitive streak, a stage where the streak extended from the posterior limits of the pellucid area nearly to its center. As Wetzel ('29) mentions, the early stage of the streak, one-fifth to one-fourth the pellucid area in length, is not often seen. Rather, the majority of the early streaks found are equal in length to at least one-third the diameter of the pellucid area.

Measurements taken at the time of operation on 213 living blastoderms in salt solution and with the yolk removed give 3.73 mm. as the average diameter of the whole blastoderm. The range in diameter is from 2.9 to 4.5 mm. Such figures compare favorably with those obtained by earlier workers. Edwards ('02) records an average diameter of 4.41 mm. His (1868) and Mitrophanow (1899) give averages around 3.5 mm., and Dehnel ('29) gives the lowest figure of 3.0 mm.

In thirty-five cases the entoderm had completed its growth anteriorly and the contour of the pellucid area was complete, showing an average diameter of 2.17 mm., with a range of from 1.51 to 2.66 mm. The figures for the pellucid area agree, in general, with the averages of 2.51 mm. (Edwards, '02), of 2.0 mm. (Dehnel, '29), and a range of 1.75 to 2.5 mm. (Wetzel, '29). Also based on these thirty-five cases, the average width of the opaque area is 0.86 mm. posteriorly and 0.74 mm. anteriorly. These figures suggest that this area is broader posteriorly than anteriorly, but in several cases, there was no measurable difference in the width of the two regions, and in other instances the difference was much smaller than indicated by the averages above. Consequently, this means of orientation for blastoderms in which the pellucid area appears homogeneous is not entirely satisfactory. Contrasting with this, Mitrophanow (1899) states that from the beginning a difference in width of the opaque area posteriorly and anteriorly presents a basis for orientation of the

disc, but Wetzel ('29) finds that often the two regions do not differ in width, which is in agreement with my findings.

OPERATIVE PROCEDURE

For all experiments, the eggs used were from S.C. white leghorns, and the donor eggs were never incubated prior to transplantation. In preliminary experiments, the unincubated blastoderm was freed of yolk in the manner described below, but was not oriented, measured, or cut. For the subsequent experiments, the procedure was as follows: the orientation of the blastoderm was determined according to von Baer's rule (1828) that the embryonic axis in a large number of cases lies perpendicular to the egg axis with the posterior end toward the observer when the blunt end of the egg shell is held to the observer's left. As a means of orientation during subsequent manipulation, the posterior end of the blastoderm was marked while still on the yolk with a very small piece of agar plate stained with neutral red (Vogt). Thus marked, the blastoderm with the yolk immediately underlying it was cut away from the remaining yolk with scissors and lifted on a section-lifter to a Petri dish of 0.9 per cent sodium chloride solution kept at 38° to 39° C. Forceps were used to remove the vitelline membrane and the blastoderm was then with the aid of steel needles gradually lifted from most of the adhering yolk and transferred in a wide-mouthed pipette to a watch glass of fresh warm saline on a warming stage. There, under the binocular dissecting microscope, the blastoderm was placed upside down and again with steel needles, the remaining yolk globules were removed with great care from the under surface of the pellucid area until this surface no longer showed any evidence of loose yolk and by far the majority of this substance was removed from the opaque area. It is impossible to get rid of all the yolk without injury to the blastoderm for much lies in the region of the germ wall and mingles intimately with the cells. Yolk granules are found, too, in the cells, as well as among the cells and between entoderm and meso-ectoderm. Thus freed

of most of the adhering yolk, the blastoderm was very carefully observed under the microscope for any structural peculiarities or any possible indications of primitive streak formation. This last precaution was very necessary, for often in warm weather all degrees of primitive streak formation, even up to nearly definitive stages, were found in these eggs, though the donors were never incubated. The term 'unincubated,' as often used, may therefore be misleading.

It must be emphasized that in these experiments, no blastoderm was used which showed any visible evidence of primitive streak formation or a pellucid area which was other than circular in outline. After such careful scrutiny, a sketch was made of each blastoderm, and it became increasingly evident that a morphological distinction between anterior and posterior ends of the blastoderm could in many cases be detected. This distinction is based on variability in the anterior extent of the entoderm. Uncertainty as to orientation when the latter is based on the somewhat variable rule of von Baer can create a serious source of error when it comes to analysis of the results. The presence in many instances of a morphological indication of the anteroposterior axis eliminates this uncertainty and provides the basis for precise location of the cuts. More reliance can thus be placed on the interpretation of the results. The oriented blastoderms were next carefully measured with the aid of an ocular micrometer and cut into pieces of known size. The measurements and the position of the cuts were then recorded on the previously made sketch of the blastoderm. The cuts were accomplished with a glass micro-needle, which instrument proved well suited to this purpose, making a precise clean cut, without the dragging and pulling of the soft tissue which occurred when steel needle-knives were tried. The pieces were transferred in a pipette of small bore adapted to the size of the piece to the chorio-allantoic membrane of the 9-day host embryo through a window already prepared in the shell. The site for the window had been previously determined by candling the host egg and marking the shell over a point at which occurred the

union of two or more large blood vessels of the chorio-allantois. After the tissue was placed on the host membrane, the piece of shell removed to form the window was replaced, its edges sealed with paraffin, and the host returned to the incubator. All grafts were allowed to grow for a period of 9 days. They were then removed, fixed in Bouin's solution, serially sectioned at 8μ , and stained with iron haematoxylin. This technique of chorio-allantoic grafting has been developed largely by Willier and his students.

GENERAL ANALYSIS OF THE GRAFTS

A table is here presented giving the frequency of appearance of the various tissues in the grafts of the different series. An examination of the table shows that epithelial tubules, non-specific in character, are present in all grafts, while smooth muscle appears with nearly as high a frequency. Gut occurs in only about half the cases and central nervous tissue in less than a third. Many other tissues are present in a smaller proportion of the grafts. Certain types of differentiation, such as proventriculus and mesonephros, representing definite levels along the anteroposterior axis of the embryo, are encountered only once. However, the occurrence of even one case proves that the unincubated blastoderm has the capacity under favorable conditions to form such organs. It is also of interest to note that while the presence of central nervous tissue does not presuppose extensive differentiation of other tissues, nevertheless, such development is not found in its absence.

A preliminary series of experiments was undertaken to ascertain whether the unincubated blastoderm had any powers of differentiation when transplanted as a whole. Nineteen grafts (15.07 per cent) were recovered out of 126 cases where the host survived. This low percentage of grafts may be partially accounted for by inexperience of the operator in the technique of chorio-allantoic grafting as well as in lack of skill in the removal of the unincubated blastoderm free from injury. It may be, though, that the whole blastoderm

does not incorporate so well as a smaller piece. In subsequent experiments, where the blastoderm was cut into various pieces, these technical difficulties were largely overcome, and visible injury to the blastoderm was rare. Consequently, the graft frequencies may for these experiments be considered more nearly as a true index of the capacity of the piece transplanted to differentiate.

In eight (42.1 per cent) of the grafts from the whole blastoderm the following tissues have differentiated, but in no one graft are they all present: gut, smooth muscle, heart, liver, epidermis, dermis, skin vesicles with feather germs, central nervous tissue (often non-specific as to level, but sometimes specific in this respect as indicated by eye and mesencephalon), ganglion cells, cartilage, lung, chorda, and mesonephros with glomeruli. In one case, proventriculus has developed as masses of glandular pockets opening from a large central cavity, and the whole surrounded by both circular and longitudinal muscle layers. The degree of differentiation attained by these various tissues is, in general, equivalent to that seen in the 9-day normal chick. Thus the experiments show that the whole unincubated blastoderm has the capacity to form, under favorable conditions, nearly all tissues typical of the embryo back to and including the level of the mesonephros.

In an attempt to locate the area, or areas, having such a capacity, the blastoderm was cut up into various pieces. The manner of cutting is indicated in figure 1.

The grafts of the first series bring out a striking disparity in capacity for differentiation of the anterior and posterior halves. The posterior halves show development of tissues essentially equal to that found in grafts from the whole blastoderm. In addition to the tissues (except mesonephros, proventriculus, and lens, here absent) found in grafts from wholes, are noted oesophagus, thyroid, sensory retina, hypophysis (?), skeletal muscle, and membrane bone. The graft frequency for posterior halves was 32.6 per cent (seventeen) of the fifty-two cases where the host survived. Eight of these

grafts showed considerable development of central nervous tissue.

The grafts from the anterior half in contrast present no such picture. Graft formation occurred in only 12 per cent (six) of the fifty possible cases. The only tissues differentiating are gut, smooth muscle, heart, and liver, except in one case where the only development consists of a mass of central

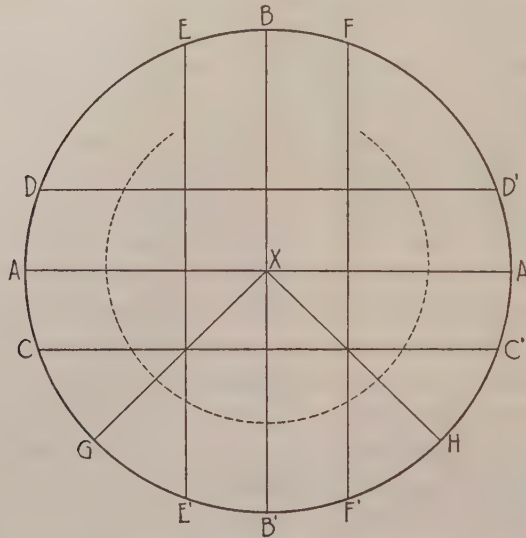


Fig. 1 Diagram of pre-streak blastoderm. The contour of the pellucid area is interrupted anteriorly. The lines indicate the location of the various cuts. 1, A-A' separates anterior and posterior halves. 2, B-B' separates right and left halves. 3, A-A' and B-B' isolate four quadrants. 4, A-A', C-C' and D-D' give four strips transverse to the anteroposterior axis of the embryo. 5, B-B', E-E' and F-F' give four strips parallel to the long embryonic axis. 6, X-G and X-H separate the median posterior quadrant from the anterior three-fourths of the blastoderm.

nervous tissue in the form of a large brain vesicle and eye. This exceptional case came from a blastoderm whose orientation could not be determined on morphological grounds.

These results led to the second series of cuts, isolating right and left halves of the blastoderm, for if posterior halves could exhibit such powers of differentiation in grafts, it might well be that both right and left halves would be equally

capable of development. There were ninety-two cases in all, forty-six for each half, where the host survived. Twelve grafts, six (13.04 per cent) from each half, were obtained. This frequency is much lower than for the posterior halves, and none of these grafts showed extensive differentiation of structures. Gut, smooth muscle, heart, liver, cartilage, epidermis, dermis, skin vesicles with feather germs developed from the left halves, and, as in grafts from anterior halves, differentiation, though specific, was never extensive. Right halves gave only gut and smooth muscle. No central nervous tissue developed, except in one graft where a few poorly differentiated tubules of nervous character were found in addition to smooth muscle, gut, heart, liver, and cartilage. This case was from a left half of a blastoderm in which morphological criteria for orientation were lacking at the time of operation.

The third series is composed of grafts from the four quadrants of the blastoderm. The left and right anterior quadrants each gave two (7.4 per cent) grafts out of twenty-seven possible cases of each type of transplant. The only tissue found, aside from the epithelial tubules, is smooth muscle. The left posterior quadrants, out of twenty-eight possible cases, gave four (14.2 per cent) grafts showing only smooth muscle and gut. The right posterior quadrants gave four (13.7 per cent) grafts out of twenty-nine cases, and while three of these showed good differentiation of tissues, it was noted that all three came from blastoderms which had been oriented only on the basis of von Baer's rule. These three grafts all show differentiation of smooth muscle, skin, central nervous tissue, cartilage, and ganglion cells. Gut, membrane bone, and lung are found in two of the grafts, while heart, a feather germ, pigmented retina, chorda, and questionable gut appear only once. Except in these three exceptional cases, gut and smooth muscle are the only tissues present in grafts from the two posterior quadrants.

Turning next to grafts of the fourth series, the blastoderm was cut into fourths transverse to the anteroposterior axis.

We find that the posterior strips gave seven (30.4 per cent) grafts out of twenty-three possible cases, and one of these grafts showed specific, but not extensive, development of central nervous tissue. The central posterior strips, bounded by the cuts A-A' and C-C', gave three (10.3 per cent) grafts out of twenty-nine possible cases and two of these gave evidence of poorly differentiated central nervous tubules. The results from grafting these two strips seem to indicate that the transverse cut dividing the posterior half tends to interfere markedly with the expression of totipotency exhibited by that piece transplanted intact. The only tissues developing from the two posterior fourths in addition to the nervous tissue are gut, smooth muscle, heart, liver (?), a feather germ, and cartilage.

The central anterior strips isolated by the cuts A-A' and D-D' gave three (12 per cent) grafts out of twenty-five possible cases and no graft showed differentiation of more than smooth muscle in addition to the omnipresent epithelial tubules. Not one graft was recovered from the most anterior strips out of twenty-eight cases with the host surviving. It seems, then, that whatever capacity for differentiation exists in the anterior half is located in the more central portion of the half. In this series, the region posterior to the center of the blastoderm has a greater capacity for graft formation and for differentiation than that anterior.

For the grafts of the fifth series, the blastoderm was cut into longitudinal fourths, i.e., parallel to the anteroposterior axis. The left strips gave two (9.09 per cent) grafts out of twenty-two possible cases, the left median strips gave three (15 per cent) grafts out of twenty possible cases, the right median strips gave three (13.04 per cent) out of twenty-three possible cases, while the right strips gave three (14.2 per cent) grafts out of twenty-one cases. The grafts of this series show differentiation of gut, smooth muscle, and heart. None of the development was extensive and no signs of any central nervous tissue were found in this group of grafts.

Up to this point, the results suggested that with the pre-streak blastoderm totipotency and extensive differentiation may be expected only when two conditions are fulfilled. First, the piece grafted must contain the posterior median region of the blastoderm; and, second, this area must remain undisturbed particularly in the vicinity of the median line.

Consequently, the sixth series of experiments was designed to test the correctness of these indications, and at the same time to provide a further check on the assumption that it was not primarily size of piece which was influencing the extent of differentiation occurring in these grafts. The median posterior quadrant was isolated from the remaining three-fourths of the blastoderm and the two pieces, so very unequal in size, were grafted. It turned out that the capacities of the two pieces were strikingly unequal, but in an inverse order, i.e., the smaller piece had a greater capacity for differentiation than the larger.

In this final series, out of forty-three possible cases, only seven (16.2 per cent) grafts were obtained from pieces comprising the anterior three-fourths of the blastoderm. With one exception, none of these grafts gave more than gut and smooth muscle. The lone exception was a case where, again, orientation was based only on the supposed perpendicular relation of the embryonic and egg axes. This one graft showed differentiation of smooth muscle, two hearts, a feather germ, cartilage, and a large brain vesicle.

Out of forty-two cases where the piece transplanted comprised only the median posterior quadrant, eleven (26.1 per cent) grafts were recovered, all from blastoderms whose orientation was absolutely certain, and of these eleven, five (45.4 per cent) contained central nervous tissue. Extensive tissue differentiation also occurred, especially in one graft (88-6), which shows development fully as extensive and specific as in any of our cases. Nearly the entire anterior end of the embryo is represented by the tissues found in this graft, described in detail below.

Thus did the median posterior quadrant express its capacity for totipotency—a capacity apparently confined to this one area of the blastoderm.

HISTOLOGY OF THE GRAFTS

First to be described are the non-specific epithelial tubules present in all grafts from every region of the pre-streak blastoderm. In some grafts, aside from a little smooth muscle, these tubules constitute the only 'differentiation,' but in other cases, though present in equal number, they form only an insignificant proportion of the tissues developed. The epithelium of these tubules is usually non-specific—occasionally it suggests sensory epithelium or gut. Smooth muscle may accompany any of these tubules. The character of the wall of such a tubule may vary markedly along its course from a simple low, to a high, or even a high stratified type of epithelium. Vacuolization of the cells is sometimes noted. By far the majority of these tubules lack specificity and are without smooth muscle. Since meso-ectoderm forms the more coherent and extensive constituent of the piece grafted, it seems logical that certain of the tubules are derived from this layer. However, some probably arise from the entoderm.

Grafts from pieces in which the intact median posterior region of the blastoderm is absent. Gut is present as branching tubes and small sacs of a characteristic epithelium. Well-differentiated smooth muscle is present as a definite circular coat about the gut. Heart appears as a compact, rounded mass of specific interlacing strands of cardiac muscle, lying almost invariably next to a coelom-like space in the mesenchyme of the host chorio-allantois. This location is characteristic not only for these, but for all, grafts. Blood spaces are rarely noted in the cardiac muscle in this group of grafts. The frequent occurrence of heart, even in the absence of the median posterior region, is not surprising, since Olivo ('28), using the unincubated chick blastoderm, obtained differentiation of pulsating cardiac muscle in tissue culture. This tissue developed from regions of the blasto-

derm peripheral to a circular area 1 mm. in diameter taken from its center. The central area never gave rise to cardiac muscle in his cultures. All pieces used in our experiments contained at least some of the peripheral areas. Liver is present in the form of clusters of small tubules usually in contact (fig. 5), or nearly so, with the heart (Willier and Rawles, '31 b). The cells of its epithelium are similar to those of the normal liver of corresponding age (9 days), dark staining, small, and with prominent nuclei. The tubules are sometimes loosely clustered, and may have a relatively large lumen. In such cases, the resemblance to the more compact cord-like arrangement of the normal is not so close. Skin, found in one graft from a left half, shows both epidermis and dermis well developed. The degree of differentiation is similar to that seen in figure 6, though keratinization of the surface layers has not advanced so far. Skin vesicles and feather germs appear in one graft, also from a left half. Epidermis forms the wall of the vesicle into whose lumen projects the feather germ, with its definite mesodermal core. In one graft a trace of typical cartilage was noted.

Grafts from whole blastoderms and from pieces containing the median posterior quadrant intact. While no higher degree of differentiation than that described above is found in some of these grafts, still there are instances (table 1) where extensive development of embryonic parts has occurred. Perhaps the description of one case will give some idea of the type of differentiation obtained. For this purpose is chosen graft 88-6, from the median posterior quadrant of a blastoderm similar as regards stage of development to that seen in figure 2. It is emphasized that this quadrant comprises only one-fourth of the blastoderm.

Central nervous system. Fore-brain is definitely represented by an eye (fig. 8) approximating in shape a normal optic cup. The pigmented layer consists of a low epithelium whose cells are relatively full of pigment granules. The transition from pigmented to sensory layer is abrupt as in the normal. The sensory retina shows the layered condition

characteristic of the 9-day normal, and in another section its inner layer gives rise to a large nerve which ends blindly in the mesenchyme. Though small, this eye is striking in its histological similarity to that of the normal embryo of corresponding age. Mid-brain is present as a large brain vesicle whose walls show the striations characteristic of the optic lobes. In this particular instance, the histology of the mesencephalon is not so pronounced as in four other grafts, where the differentiation (fig. 10) compares favorably with that of the 7-day normal. Nervous tissue, non-specific as to level, is also present in the form of large brain vesicles with thick walls of gray and white matter. Nerves lead off from the latter to end blindly. In one region there is a considerable mass of unorganized nerve cells and fibers heaped together with very irregular boundaries. Clusters of absolutely typical ganglion cells are found occasionally along the course of nerves or close to the main mass of nervous tissue.

Skin. A large area of epidermis and dermis (fig. 6) quite typically differentiated is present. A feather germ with its well-vascularized dermal core has developed in a skin vesicle apparently independently of the large area of skin. Both skin and feather germ show a development definitely accelerated as compared to that of the control-age embryo—a situation common for these two organs in all our grafts.

Entodermal derivatives. Oesophagus is present, its epithelium characteristically folded and surrounded at a short distance with a circular coat of smooth muscle. Close by lies trachea, with a condensation of mesenchyme, probably pre-cartilaginous, around its stratified epithelium. Near the heart is a large mass of liver tissue (Willier and Rawles, '31 b). The histology of the latter is similar to that described for the grafts of the first group. The liver opens into a small duct whose epithelium corresponds to that of the gall bladder, and this in turn opens into some gut tubules surrounded by circular muscle. One of these tubules loses its muscle coat, enlarges, and takes on the character of pharynx. From this region is budded off a small area of thyroid whose glandular

epithelium forms compact cords bounded by numerous capillaries. No colloid is present. This thyroid is roughly similar in its histology to that of the 9-day chick, and also to the cord-like type obtained by Rudnick ('32) in grafts from blastoderms of definitive streak and head-process stages. However, there is not evident in this case the acceleration noted in some of her grafts from the definitive streak blastoderm.

Mesodermal derivatives. Smooth muscle, cartilage, cardiac muscle, and notochord, all typically developed, are found. The cardiac muscle forms a large heart, connecting with blood vessels of the host membrane by way of numerous interconnecting blood spaces lined with endothelium—a condition frequently encountered in this group of grafts. While there is no external limiting membrane about the heart muscle, the latter is absolutely distinct from the surrounding mesenchyme.

The case just described may be considered typical of the degree of differentiation attained when the intact median posterior region is present in the piece grafted, yet certain peculiarities may be noted. Development of the eye and regional differentiation in the gut have gone further than in any other graft. However, in other grafts, there may be less unorganized central nervous tissue and certain tissues unrepresented in this type case are found. These tissues are typical membrane bone and lung, represented by a low epithelium similar to the normal, with its characteristic nuclei. This epithelium is not always organized into such definite tubes as in the normal, but tends to appear in more cord-like masses. Striated muscle is present, associated with cartilage, in one graft. A mass of folded, glandular epithelium in close contact with the wall of a brain vesicle is found in another instance and identified as probable hypophysis. Mesonephros, too, occurs once, in the form of a cluster of specific tubules about three distinct glomeruli. An abortive lens (fig. 9) appears once in conjunction with a large vesicular eye formed in part of pigmented epithelium. This lens is continuous on one side with the pigmented epithelium and on the other with skin. Mangold ('31) finds the weight of evidence in Amphibia

against the induction of lens by pigmented layer of the retina. His summary of the work on the induction of lens by optic cup in birds indicates that the details are as yet uncertain. Working on the chick, Danchakoff ('24) speaks of typical lens formed, in chorio-allantoic grafts, from ectoderm not only when the eye development is normal, but even when groups of pigmented cells have developed and come into contact with ectoderm. This seems to be the situation in this graft. Danchakoff further ('26, p. 78) comments on the "necessity for an intimate and extensive contact between the two primordia" for lens formation. That lens was obtained only once is not astonishing, for there are apparently many conditions to be fulfilled, and the chances of all occurring must be slight in grafts from younger stages. Even in older stages, lens occurs only infrequently (Willier and Rawles, '31 a).

Not all parts in a given graft are equally well developed, and even in the case of one particular tissue, it may be present both well and poorly differentiated. The combinations of tissues vary and would rarely be exactly the same in any two grafts. The number of specific tissues in the graft described, while greater than in some, is equal to, though not exceeded by, that in several others. Central nervous tissue may be present in these grafts as unorganized masses of cells and fibers, as large or small brain vesicles non-specific as to level (fig. 7), or as definite levels along the embryonic axis indicated by fore-brain (eye) and mesencephalon. All three grades of differentiation, any two, or only one of them may be present in a given graft. Hind-brain and cord have never been identified in our grafts, though they may be represented. Likewise, gut may be present non-specific as to level, or distinct regions within the system may be indicated by the presence of any or several of the following tissues: oesophagus, trachea, lung, pharynx, thyroid, gall bladder, and liver. As with the central nervous tissue, so with the gut, more than one, or only one, grade of organization may be found within a single graft.

Nearly all the tissues representing the anterior end of the embryo back to, and including, the level of the mesonephros have been found in grafts from pre-streak blastoderms, entire and in pieces. This situation is likewise characteristic of grafts from whole blastoderms (Willier and Rawles, '31 a), or from pieces containing the node level (Hunt, '31, '32), of the definitive streak blastoderm. The significance of the situation is discussed in the paper of Willier and Rawles ('31 a).

ANALYSIS AND DISCUSSION

The results of the experiments just described demonstrate quite strikingly that, with six exceptions, one area alone of the pre-streak blastoderm has the power to differentiate extensively in the chorio-allantoic graft. This area is the median posterior quadrant of the blastoderm. Grafts from wholes, or from pieces which include this area intact, show differentiation of nearly all tissues representing the anterior end of the embryo back to, and including, the level of the mesonephros. On the other hand, pieces lacking this region are apparently incapable of giving rise to any such development. No central nervous tissue is found, and the tissues typically differentiating in such grafts are small areas of gut, smooth muscle, heart, liver, and skin. In agreement with these results, Umanski ('31) describes a few cases of chorio-allantoic grafts from pre-streak and broad primitive streak stages where the whole blastoderm or posterior halves gave differentiation of central nervous tissue and mesodermal elements, while grafts from anterior halves failed to show any development.

This localization of the capacity for differentiation of embryonic parts suggests that the organization of the embryo is centered about the median posterior area of the pre-streak blastoderm. In other words, the blastoderm is definitely polarized, and while, morphologically, there is no particular evidence of this condition, yet, physiologically, there must be a very marked organization to admit of such striking disparity in the results obtained from grafting pieces with and without the intact median posterior region of the blastoderm.

Moreover, this area grafted alone is fully as capable of giving rise to embryonic parts as is the whole blastoderm. The experiments of Gräper ('29) and Wetzel ('29) indicate that, in general, it is the posterior region which is normally concerned in the formation of the embryo, and the work of Hyman ('27) suggests that for a blastoderm incubated 7 hours this general area is relatively more active, as indicated by susceptibility experiments, than the remainder of the blastoderm.

A postero-anterior gradient in developmental capacity is indicated by the results of experiments cutting the blastoderm into four transverse strips. The posterior strips gave grafts in 30.4 per cent of the cases, the central posterior strips in 10.3 per cent, and the central anterior strips in 12 per cent. No grafts were recovered from the anterior strips. The posterior region apparently has the greatest and the anterior region the least capacity for differentiation as shown by these grafts. Both central strips show an intermediate frequency of graft formation. However, the central posterior strips give rise to several tissues, while the central anterior strips develop only smooth muscle in addition to the epithelial tubes. Thus even between these two central strips there is a developmental gradient favoring the more posterior. Further, a comparison of the graft frequencies for the two anterior transverse fourths with that obtained for the anterior halves transplanted in one piece (12 per cent) reveals that whatever capacity for differentiation is present lies in the more central part of the anterior half.

There is a higher frequency of graft formation from pieces containing some of the posterior area of the blastoderm as compared with pieces where only anterior regions are represented. The posterior pieces are grouped to include all those containing any of the posterior region, with and without the median area intact. The host survived in ninety-eight cases where known posterior pieces were grafted, and twenty-three grafts (23.4 per cent) were recovered, while from 100 similar anterior cases only eight grafts (8 per cent) were obtained.

This difference is statistically significant, showing a markedly lower capacity for differentiation in pieces with only anterior, as opposed to posterior, regions of the blastoderm.

While the fifth series of our grafts does not suggest a medio-lateral gradient in developmental capacity, nevertheless, the results on the whole indicate that the presence of the undisturbed median region is essential for extensive differentiation of embryonic parts. Perhaps, if lateral pieces had been compared with intact median strips, rather than with longitudinal halves of such strips, a medio-lateral gradient might have been evident.

It was mentioned at the beginning of this discussion that six exceptional cases were found, all from blastoderms oriented only on the basis of von Baer's rule, which is known to be variable. Von Baer (1828) himself states that there may be variation to either side of the perpendicular, and later authors find essentially the same situation. The perpendicular relation of the two axes is found in only 33 per cent of the cases by Kopsch ('27), in 75 per cent of the cases by Duval (1884), and others present figures of intermediate range. Deviation is, however, not great, as most cases are rotated only slightly to either side of the perpendicular. The percentage of cases inverted varies from 0.6 per cent (Duval, 1884) to 33 per cent (Bartelmez, '18), while Kopsch ('27) gives 5.8 per cent and Dalton (1881) places it at 12 per cent. Since authors give figures which vary so widely, it may be that different groups of eggs show characteristic differences in range of variability. Therefore, a check was undertaken on the material used in these experiments, and the following information was obtained based on the orientation of 160 eggs.

With the blunt end of the egg shell to the observer's left, according to von Baer's rule, the anterior end of the blastoderm should be directed away from the observer. This condition was found in only 50 per cent of the cases examined; 33.1 per cent of the eggs showed the anterior end of the embryonic axis rotated 45° or less to the observer's right, and

2.5 per cent rotated more than 45° in this direction; 10.6 per cent of the eggs showed an axial rotation of 45° or less, and 2.5 per cent a rotation of more than 45° , to the left of the perpendicular. Inversion, where the axes are approximately at right angles, but with the anterior end of the blastoderm facing the observer, was encountered in 1.25 per cent of the 160 cases.

Such figures emphasize the variability in axial relations found in these eggs, with von Baer's rule holding for only 50 per cent of the cases. Since orientation in the six exceptional cases was based solely on this rule, it seems perfectly justifiable to class these as instances where the embryonic axis has rotated with reference to the egg axis so that the intact median posterior region is actually included in the piece grafted. Out of 435 cases, with viable hosts, where pieces lacking this area were grafted, only these six cases (1.37 per cent) were obtained. In view of the high percentages of variability in axial relations, such an explanation seems probable. That more exceptions were not encountered is puzzling. However, it must be borne in mind that the preliminary orientation based on von Baer's rule was subsequently checked by a morphological examination. Since deviation from the perpendicular is usually slight, in those cases where morphological criteria were absent, the cuts may have been close enough to the true median line to interfere with developmental processes.

This explanation is further strengthened by a consideration of those cases where the orientation of the blastoderms grafted was definitely known. In eight out of fifteen grafts (from forty-four trials in which the host was alive) where the piece grafted contained the median posterior quadrant intact, central nervous tissue differentiated. In fact, all kinds of tissues (except skeletal muscle) listed in the table as occurring in grafts from posterior halves and median posterior quadrants are likewise found in these eight cases. This is to be contrasted with twenty grafts (out of 189 cases in which the host was living) obtained from any type of piece lacking the

intact median posterior quadrant, and in which central nervous tissue failed completely to differentiate or other tissues fail to develop extensively. In these cases only very small amounts of the following tissues are found: smooth muscle, gut, heart, epidermis, dermis, and cartilage.

At this point, certain results obtained by other authors may be compared. Prior to early head-process stages, Danchakoff ('24) found no differentiation in chorio-allantoic grafts from blastoderms either whole or cut into pieces. She considers as most unfavorable the highly modified form of nutrition to which the younger stages must become accustomed when transferred from the yolk to the host chorio-allantois. Danchakoff may not have grafted a sufficient number of these younger stages to get a true index of their capacity. As for the pieces, the cuts may have been so located as to disturb the median posterior area of the blastoderm, thus inhibiting development.

Murray and Selby ('30) grafted unincubated blastoderms both whole and in pieces. In the case of the whole blastoderms, they obtained considerable differentiation of tissues. In their grafts from pieces representing intended transverse levels of the unincubated blastoderm, they obtained differentiation of central nervous tissue from a few pieces coming supposedly from central and anterior regions. However, they mention specifically for one of these cases, as well as in general, that orientation is not always certain, since it is hard to keep track of this during manipulation subsequent to removal of the blastoderm from the yolk. They further comment on the uncertainty as to the exact stage used, so they may have used streak stages. Such uncertainty makes it hard to regard their results as conclusive.

Turning to evidence from other sources bearing on our problem, it is interesting to compare the limits of this median posterior area, which according to our experiments is alone capable of differentiating extensively, with the extent of the presumptive embryonic areas as described in maps constructed on the basis of methods other than grafting. Gräper ('29)

and Wetzel ('31) present such maps projected on the round pellucid area of the blastoderm prior to the appearance of the streak. Waddington ('32) offers a third map, in which he combines certain features of the first two with modifications of his own. In details, of course, the three maps differ, but all agree in placing the bulk of the presumptive embryonic areas, with the exception of much of the area destined to form epidermis and a very small portion of the presumptive neural region, in the posterior half of the pre-streak blastoderm. Furthermore, both Wetzel and Waddington place the majority of the presumptive areas more median than lateral. Thus the median posterior quadrant could easily include at least a good sample of all the presumptive areas. Evidently, as is indicated both by our grafts and by these maps, this region is active in the formation of the embryo.

Also of considerable interest is the close similarity in the limits of the median posterior region to those of the area which Gräper ('29) and Wetzel ('29) consider most actively concerned in the formation of the primitive streak. Gräper studied a series of moving pictures of vitally-stained living embryos, and Wetzel traced the movements of vitally-stained areas of early blastoderms. Though differing with respect to certain details, both Gräper and Wetzel agree, in general, on the cell movements leading, during the early hours of incubation, to the formation of the primitive streak from the material lying in the more posterior region of the pellucid area. These movements center about the median line and are first noticeable and most prominent in the posterior half of the blastoderm. As the material streams forward in the median line of the future embryo, it is replaced by material sweeping into the posterior area from the postero-lateral and lateral regions. The anterior movements in turn displace material which sweeps laterally and then joins in the posterior movements along the lateral margins of the pellucid area.

Our results indicate that the median posterior region must be grafted intact if it is to retain its power for differentiating parts of the embryo. Thus when a cut is made in, or close to,

the median line of the blastoderm, no differentiation of central nervous tissue or extensive development of tissues occurs even from the posterior region. Apparently, the combined action of the areas to either side of the median line, or at least the presence of the median region undisturbed, is essential for such differentiation.

It is rather striking that this inhibition is apparently caused by just those cuts, i.e., in the median line, which would seemingly interfere most with the cell movements described above. In fact, any cuts in the posterior region might well be disturbing. Thus with the transverse fourths of the blastoderm, the only grafts showing differentiation of central nervous tissues came from pieces of the posterior half of the blastoderm, and this differentiation was never extensive. The cut along the line C-C' might leave the region destined to form the anterior end of the streak in the posterior, or the central posterior, strip depending on the exact level of the cut. Or, variation in the exact age of the blastoderm might on the basis of these cell movements have the same effect. In any case, there would probably be considerable disturbance of the area, and this might explain the low number of grafts, from the posterior fourths, showing differentiation of central nervous tissue or extensive development.

The capacity to form parts of the embryo seems to reside primarily in that region of the pre-streak blastoderm which is most actively concerned in primitive streak formation, i.e., the median posterior quadrant. Further, any cut which would tend to interfere with the cell movements leading to primitive streak formation also apparently lowers the capacity of the piece for development. Whether there is actually completion of these cell movements, or formation of a primitive streak, in the piece grafted, is a question which cannot be answered by these experiments.

The material for the final section of the discussion is provided by an examination of certain generalizations. It might have been expected that blastoderms of pre-streak stages would show less specific or less extensive differentiation than

those with streaks, but such differences in developmental capacity between these stages were not found. Wholes or pieces containing the intact median posterior region of the pre-streak blastoderm may exhibit differentiation nearly as extensive and fully as specific as does the whole blastoderm (Willier and Rawles, '31), or the node level (Hunt, '31, '32), of the definitive streak stage. The only tissues not identified in our grafts from blastoderms of pre-streak stages, yet obtained from those of the definitive streak stage, are hind-brain, cord, epiphysis, cartilage bone, and epithelium of the oral cavity. Moreover, sometimes we find that grafts from blastoderms of streak stages may give little or no differentiation. In other words, under favorable conditions, an intact median posterior quadrant from a pre-streak blastoderm is practically equivalent to a piece from the node level of the definitive streak blastoderm as regards capacity to differentiate specific tissues.

In contrast to these results, Hoadley ('26 a, b, c), as a result of grafting transverse thirds and fifths of the blastoderm in stages from, and including, the unincubated stage up through that of 10 hours' incubation, concludes that an increase in differentiating capacity paralleling an increase in the age of the donor is indicated. Similarly, in other experiments ('26 d), he finds a higher degree of differentiation from older, as contrasted with younger, operative stages. He argues that such results show that the process of segregation is halted coincident with, and as a result of, grafting. Thus the progressive increase in number and specificity of the tissues indicates an orderly increase in physiological organization of the blastoderm correlated directly with the age of the donor.

It is true that the frequency of graft formation increases directly with the age of the blastoderm. Comparing pieces from pre-streak and streak stage blastoderms, we find that the former are less likely to realize their full potencies or even to develop at all. For another series of experiments not reported here, a graft frequency of 43 per cent was obtained using early streak stages, contrasted with never more

than 32.6 per cent as reported above for pre-streak blastoderms. Hunt ('31) obtained a graft frequency of 73 per cent for pieces from the node level of the definitive streak stage. Furthermore, Hyman ('27) states that younger stages of the chick blastoderm are far more sensitive to disturbance than those older. The operation as a disturbing factor may thus more frequently check organization in blastoderms of the youngest stages used, but such inhibition is not a necessary or constant accompaniment of grafting.

The lack of differentiation obtained by Hoadley from blastoderms of the younger stages has two possible explanations. First, since he does not present a very large number of grafts, the results may show merely that insufficient cases were examined on the basis of the low graft frequency for these stages. Second, with the transverse fifths, the cuts would be very close together, and with the unincubated and early streak blastoderms, such disturbance of the posterior area would tend to inhibit differentiation, as was seen in our results from transverse fourths. This disturbance would not be effective in the older stages used, for even a very narrow transverse strip from the node level of a definitive streak stage has a high capacity to develop (Hunt, '31, '32).

The presence in our grafts, from pieces of pre-streak blastoderms, of such structures as a pulsating heart in a graft from a posterior half, and in a graft from a median posterior quadrant of a layered retina histologically equivalent to that of the 9-day normal, does not argue for an arrest of the organization of the blastoderm. The difference between pre-streak, early streak, and definitive streak stages seems to lie in the increasing frequency with which grafts showing extensive differentiation are obtained from blastoderms of the older stages.

Our grafts do not offer much evidence as to localization of organ-forming potencies along the anteroposterior axis of the blastoderm. We can only say that for the pre-streak blastoderm the median posterior region has the greatest power to produce embryonic parts. Even the anterior structures

develop from the posterior, rather than the anterior, half. In contrast, Hoadley's experiments ('26 d), with the blastoderm cut 'in situ' into anterior and posterior halves, led him to conclude that there was a localization of potencies at definite levels along the anteroposterior axis of blastoderms incubated 2 or more hours, and even of unincubated blastoderms. He recognizes that the actual location of the cut with reference to the axis of the blastoderm is uncertain, and that the exact stage cannot be detected on the yolk. Since I have always found considerable variation in the stage reached in unincubated blastoderms even after keeping the eggs a day or so at room temperature, it seems probable that in some of Hoadley's cases the blastoderms may have developed as far as a moderately advanced streak stage, and consequently the anterior end of the streak may have been included in the area anterior to the cut. In such a case, as Wetzel ('29 b) has shown for the definitive streak stage, development proceeds on both sides of the cut (compare Hunt, '32).

In this connection, two possible interpretations must be recognized. First, the actual potencies of the median posterior quadrant may be greater than its prospective significance. That is, the various potencies may be localized over a greater extent of the pre-streak blastoderm, but only weakly determined. Or, localization of the potencies may not have occurred at the time of operation. In either case, reconstitution may then occur in the piece comprising the more active region, i.e., the median posterior quadrant, which behaves as a whole. Second, the potencies and the prospective significance of this region may coincide. This area may actually, in normal development, give rise to the majority of the parts of the embryo. The maps (Wetzel, '31; Waddington, '32) of the presumptive embryonic areas favor this view. However, the nature of our experiments does not enable us to decide between these alternative interpretations.

That size of piece is here not a determining factor in the capacity to differentiate is well illustrated in the contrast between the piece comprising the median posterior quadrant,

i.e., only one-fourth of the blastoderm, giving essentially all the tissues obtained from the whole, and the remaining three-fourths of the blastoderm showing little or no differentiation. Furthermore, anterior and posterior halves, with the pieces the same size, exhibit very different graft frequencies, 12 per cent for the anterior halves and 32.6 per cent for the posterior halves, with the greatest power to differentiate embryonic parts limited to the posterior halves. The degree of differentiation attained in grafts of whole blastoderms, posterior halves, and median posterior quadrants is, in general, the same, both as regards number of tissues and specificity of their development. Therefore, within the limits of these experiments, size of piece does not appear to be decisive.

In contrast, Murray and Selby ('30) conclude on obtaining less differentiation from transverse thirds and fourths than from whole unincubated blastoderms, that differentiation is inhibited following isolation of the piece from the whole. They also consider it probable that the degree of differentiation bears an inverse relation to the size of the piece. Our results fail to support these conclusions. Rather, the lack of differentiation obtained in their grafts from pieces may have been due to general unfavorable conditions. Perhaps, too, the cuts were so placed, orientation and stage being uncertain, as to interfere with the development of the median posterior region in many of their cases. Or, the number of grafts may have been too few to ascertain the full capacity of the pieces, since the graft frequency for these pieces under the most favorable conditions is low. On the other hand, a combination of such factors may have led to their results.

SUMMARY

1. In preliminary experiments, unincubated chick blastoderms were grafted entire to the chorio-allantoic membrane of a 9-day host embryo and allowed to develop for 9 days. The grafts recovered often showed a differentiation of numerous embryonic structures.

2. Exact orientation of the unincubated, pre-streak blastoderms is possible in many instances because the entodermal layer is often incomplete in the anterior part of the pellucid area. Such a morphological criterion of orientation is distinctly valuable as a basis for precise location of the various cuts in the blastoderm, since deviation from von Baer's rule is found to occur frequently. A study of the orientation of 160 blastoderms with reference to the egg axis reveals that in only 50 per cent of the cases does it agree exactly with this rule.

3. In subsequent experiments, the unincubated, pre-streak blastoderm was first oriented according to von Baer's rule, and the posterior end marked while still on the yolk with a small piece of agar plate stained with neutral red. After removal of the yolk, orientation was, in every case possible, made absolutely certain on the basis of the morphological evidence provided by the entoderm. Various cuts were then made in the blastoderm with reference to the anteroposterior axis. The resulting pieces were grafted to the chorio-allantois of a 9-day host.

4. The majority of grafts from pieces show little or no differentiation of tissues, but some exhibit considerable development of embryonic parts. Analysis reveals that extensive differentiation occurs only when the piece grafted contains intact the median posterior quadrant of the blastoderm. Nearly all the tissues of the anterior end of the embryo are represented in such grafts.

5. This one area having a high capacity for development is compared to the region mapped by Gräper ('29), Wetzel ('31), and Waddington ('32) as including most of the presumptive embryonic areas. It is further compared to the region described by Gräper ('29) and Wetzel ('29) as showing the greatest activity in the early stages of primitive streak formation.

6. Grafts from the median posterior quadrant of the blastoderm show as many tissues and as extensive differentiation as do those from posterior halves, or whole blastoderms.

Thus size of piece does not seem to be decisive within these limits. Moreover, the degree of differentiation attained is essentially identical with that obtained from the whole blastoderm or the node level of the definitive streak stage (Willier and Rawles, '31; Hunt, '31, '32), though the graft frequency in the latter stage is higher than that for the pre-streak blastoderm. In other words, in comparing blastoderms of pre-streak and definitive streak stages, there is not necessarily a direct relation between the of age of the donor and the degree of differentiation reached.

7. The results of these experiments show a polarized pre-streak blastoderm with a gradient in developmental capacity from posterior to anterior. Organization is centered about the median posterior quadrant of such a blastoderm. This region possesses the greatest capacity to differentiate embryonic parts, and thus may be compared to the node field of the definitive primitive streak stage.

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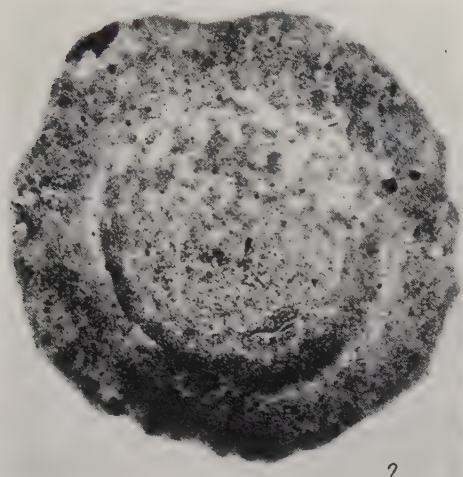
PLATE 1

EXPLANATION OF FIGURES

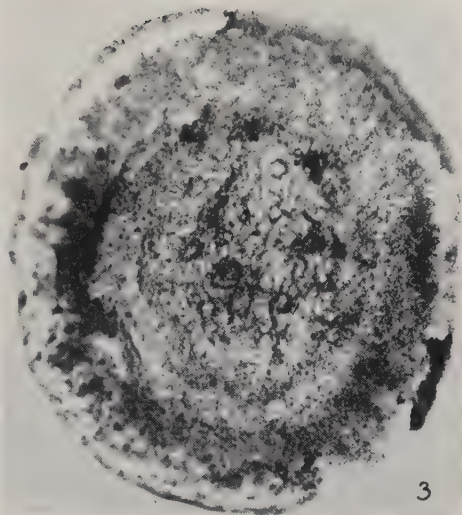
2 Unincubated blastoderm, showing the presence of entoderm as an incoherent layer anteriorly. The pellucid area has a definite contour only posteriorly and laterally. Initial $\times 23$.

3 Unincubated blastoderm with entodermal layer completed. Pellucid area appears homogeneous. Orientation unknown. Initial $\times 23$.

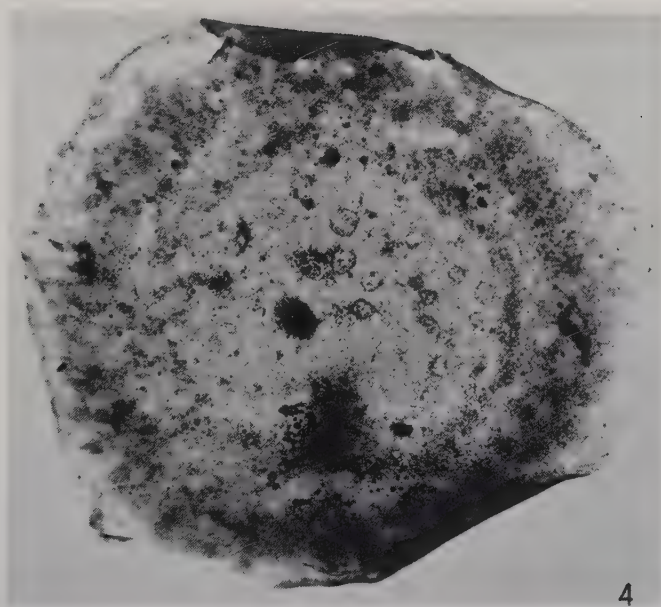
4 Blastoderm showing an early stage in the formation of the primitive streak. The short, broad streak lies at the posterior end of the pellucid area and measures one-fourth of this area in length. Initial $\times 23$.



2



3



4

PLATE 2

EXPLANATION OF FIGURES

5 Photomicrograph of liver cords in contact with heart muscle in a graft from a whole unincubated blastoderm. $\times 440$.

6 Skin appearing in a graft (88-6) from the median posterior quadrant of a pre-streak blastoderm. Epidermis and dermis are well developed. $\times 210$.

7 Central nervous tissue as differentiated in a graft from the median posterior quadrant of a pre-streak blastoderm. Note brain vesicles, nerve fibers, and irregular masses of nerve cells. $\times 35$.

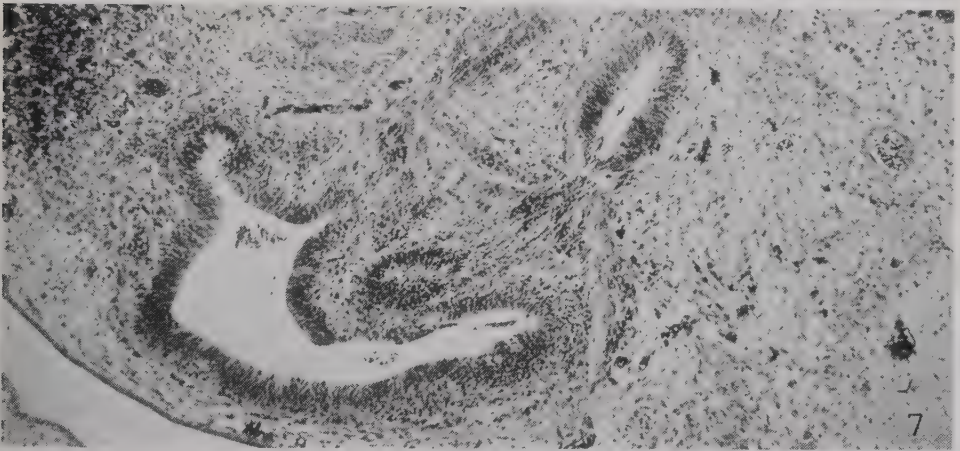
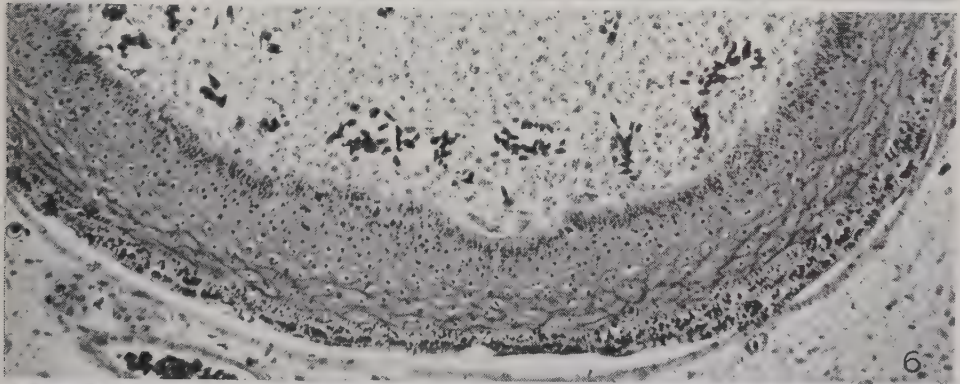
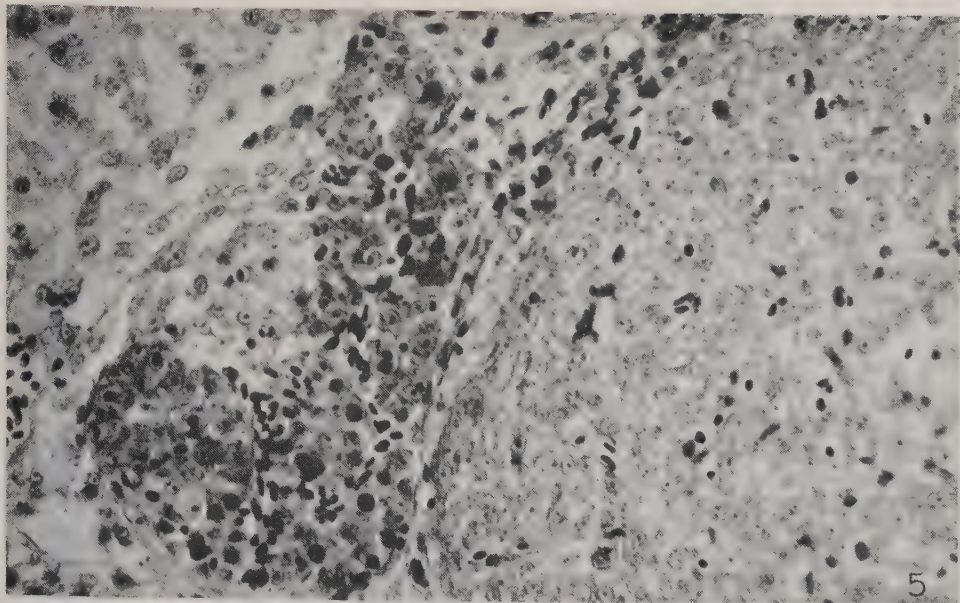


PLATE 3

EXPLANATION OF FIGURES

8 Eye developed in a graft (88-6) from the median posterior quadrant of a pre-streak blastoderm. See text for details. Initial $\times 50$.

9 Abortive lens formation in graft (26-26) derived from a whole unincubated blastoderm. See text. Initial $\times 300$.

10 Typical mesencephalon as differentiated from a whole unincubated blastoderm. Initial $\times 350$.

